cigarette smokers, using allopurinol to inhibit XO.

Methods: Fourteen cigarette smokers with a >20 pack year history (6 males; 42±3 yrs) and 14 age and gender matched healthy controls (6 males; 41±3 yrs) participated in a double-blinded, randomized, two-phase crossover study. Subjects received either a single oral dose of 600 mg of allopurinol or nothing. Endothelium-dependent responses were assessed by forearm blood flow responses to intra-arterial acetylcholine and bradykinin. Endothelium-independent responses were tested with nitroprusside.

Results: Baseline parameters were not significantly different between smokers and controls, except for HDL-cholesterol (42±2 mg/dl and 58±4 mg/dl respectively; p<0.05) and carboxyhemoglobin (4.2±0.5% and 0.9±0.2% respectively; p<0.001). After oral allopurinol, plasma oxypurinol levels reached 9.9±1.5 μg/ml in smokers and 8.2±0.5 μg/ml in controls at 7 hrs (p=0.55), and uric acid levels decreased significantly in both smokers (4.5±0.6 mg/dl to 3.6±0.6 mg/dl; p<0.05) and controls (5.0±0.4 mg/dl to 4.1±0.3 mg/dl; p<0.05). In the absence of allopurinol, forearm dilatation to acetylcholine (30 μg/min) was significantly impaired in smokers (254±57%) vs. healthy controls (390±55%; p=0.04). Allopurinol substantially increased responses to acetylcholine in smokers (463±78%; p=0.001), which approached the levels in controls (401±80%). Bradykinin responses were improved similarly to acetylcholine in smokers. Allopurinol did not improve responses in controls. Responses to nitroprusside were not significantly different between smokers (387±52%) and controls (437±36%), and were not altered by allopurinol.

Conclusion: Xanthine oxidase contributes importantly to endothelial dysfunction in cigarette smokers.

1128-88 Insulin Resistance Impairs Endothelial Function by Enhancing Oxidation of Low Density Lipoprotein in Healthy Young Men

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Background: Both insulin resistance and LDL oxidation play important roles in the pathogenesis of atherosclerosis. Recent studies have suggested that there is a significant link between these factors. In this study, we investigated the relationship between these factors and endothelial function in healthy young men, because endothelial dysfunction is regarded as an early step in the development of atherosclerosis.

Methods: Thirty-three men (aged 28.0±2.5 years), who had no history of any chronic diseases including diabetes mellitus, were enrolled in this study. We evaluated endothelial function estimated by flow-mediated vasodilation during reactive hyperemia, using high-resolution ultrasound Doppler echocardiography. We also measured plasma oxidized LDL (oxLDL) level by a sandwich ELISA method. Insulin sensitivity was estimated by homeostasis model assessment insulin resistance (HOMA-IR). HOMA-β-cell function was used to assess pancreatic insulin function.

Results: The plasma oxLDL level was significantly correlated with the body weight (r=0.436, P<0.05), body mass index (r=0.459, P<0.01), LDL-cholesterol (r=0.386, P<0.05), and HOMA-β-cell function (r=0.339, P<0.05). Furthermore, HOMA-β-cell function was significantly correlated with endothelium-dependent flow-mediated vasodilation (r=-0.376, P<0.05). When we divided these subjects into two subgroups according to HOMA-IR level (insulin-resistant group with a HOMA-IR≥2.5 and insulin-sensitive group with a HOMA-IR<2.5), the plasma oxLDL level and HOMA-β-cell function were significantly correlated with flow-mediated vasodilation (r=-0.568 and -0.521, P<0.05; respectively) in insulin-resistant group. In contrast, insulin-sensitive group showed no significant relationship between these parameters and flow-mediated vasodilation. A stepwise multiple regression analysis in insulin-resistant group showed that the plasma oxLDL and insulin levels were determinants of flow-mediated vasodilation (R^2=0.503).

Conclusions: These results suggest the possibility that insulin resistance enhances oxidation of circulating LDL particles and thus impairs endothelial function even in healthy young men.

1128-89 Immunization With a Novel Human Apo B100 Related Peptide Reduces Atherosclerosis and Inflammation in Apo E Null Mice

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Background: Immunization with homologous LDL reduces atherosclerosis in animals. To develop a clinically testable strategy, we sought to design and test human apoB100 related peptides as immunogens.

Method: A polypeptide library of 20 amino acid peptides covering the human apoB sequence was screened. Two peptides were subsequently tested using patient and normal sera, and identified as potential immunogens. Six-week old apoE null mice were then immunized with 33 μg of P1 (n=10) or P2 (n=10) with Alum as adjuvant, followed 3 weeks later by a booster. Controls received Alum alone (N=9). Mice were fed high cholesterol chow from 11 weeks of age until sacrifice at 25 weeks. Oil red-O stain of en-face prepared aorta was used to assess extent of atherosclerosis. Macrophage immunoreactivity and trichrome staining were used to assess and compare plaque composition in the aortic sinus.

Result:

Table 1: Aortic plaque size and aortic sinus lesion composition.

Group	Aortic Plaque Area	Macrophage	Collagen
Alum	20±5%	6.6±2.3%	32.3±5.3%
P1	17±4%	13.6±5.3%*	35.6±8.5%
P2	6±2%*†	1.3±0.6%*†	39.9±7.7%*

*p<0.05 vs. Alum; †p<0.05 vs. P1

Mean plasma cholesterol levels were greater than 1000 mg/dl in all groups except P1 group (715 mg/dl).

Conclusion: Immunization with specific human apoB100 related peptide P2 markedly reduced aortic atherosclerosis, reduced inflammation, and altered plaque composition in apo E null mice despite severe hypercholesterolemia raising the possibility of future clinical testing.

1128-90 Selective Expression of Tenascin in the Coronary Artery and Its High Affinity Interaction With LDL May Account for Lipid Retention Capacity of Coronary Vessels and Their Susceptibility to Atherosclerosis

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Background: Several studies have shown that internal mammary artery (IMA) in its native position as well as when grafted into coronary circulation is resistant to atherosclerosis even in patients with extensive atherosclerosis elsewhere. To understand the molecular basis for the athero-permissive nature of the coronary artery and athero-protective properties of mammary artery.

Methods: We have used porcine coronary and mammary arteries, and suppression subtractive hybridization (SSH) to generate profile of artery-specific gene expression. From a 3000 clones SSH-cDNA repertoire, we have screened the libraries by dot blot array and sequenced 2000 promising artery-specific clones. Northern blot and in-situ hybridization confirmed the differential gene expression pattern identified by the array. Cluster analysis of the sequences revealed that extracellular matrix genes that are involved in lipid retention and metabolism are predominantly expressed in the coronary artery. We hypothesized that, unlike IMA, the extracellular matrix of the coronary artery retains lipids. Therefore, we examined the interaction between tenascin, a coronary-specific gene, and LDL and minimally oxidized LDL (mo-LDL).

Results: Both LDL and mo-LDL exhibited concentration-dependent saturable binding to tenascin with similar apparent Kd values of 4.2 ± 2.6 and 5.1 ± 1.2 nM, respectively. The Bmax for ox-LDL was about 55% less than that for LDL, 28.4 ± 4.9 and 65.8 ± 12.6 fmol, respectively. Additional experiments showed that at physiologic concentrations, Ca2+ and Mg2 increase LDL binding to Tenascin 2.3- and 1.9-fold respectively.

Conclusion: Taken together with the preferential expression of other lipid binding proteins in the coronary artery, the expression of tenascin and its high affinity binding to LDL raises a possibility that unlike mammary artery, coronary arteries have an intrinsic capacity to retain lipids. This may partly explain the athero-permissive nature of coronaries.

1128-91 Inhibition of Atherosclerosis in Apo E Null Mice by Immunization With Native and MDA-Modified Apo B Peptide Sequences

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LDL oxidation is believed to have an important role in the development of atherosclerosis. Oxidized LDL has been shown to activate antibody-mediated immune responses. Experiments in which animals have been immunized with oxidized LDL suggest that these immune responses inhibit atherosclerosis. Epitopes in oxidized LDL that induce antibody formation in man was identified by ELISA using a library of native and MDA-modified polypeptides covering the complete apo B sequence. The ability of these epitopes to induce athero-protective immune responses was then analysed in apo E null mice. Mice were immunized at 6 and 9 weeks of age with 100 μg of (A) MDA-modified peptides against which high IgG levels were detected in CHD patients, (B) native peptides against which high IgG and IgM levels were detected in healthy controls and (C) MDA-modified peptides against which high IgG levels were detected in healthy controls, using Alum as adjuvant. The mice were given a high cholesterol diet from 10 weeks of age and sacrificed at 25 weeks. Immunization with peptides B and C resulted in a significant inhibition of atherosclerosis as assessed by Oil Red O staining of flat prep aortas (0.102±0.055% and 0.124±0.086% lesion area) as compared to controls given adjuvant alone 0.287±0.071%; p<0.0001). Immunization with peptides A did not influence lesion formation (0.338±0.079%). Cholesterol levels were not affected by immunization with any of the peptides. These studies have identified peptide sequences in oxidized LDL that induce immune responses that inhibits atherosclerosis and suggest the possibility of developing an immunization therapy for CHD.

1128-92 LOX-1 Mediates Oxidized LDL-Induced the Expression of Matrix Metalloproteinases in Human Coronary Artery Endothelial Cells

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Background and objectives: Increasing evidence indicates that oxidized low-density lipoprotein (ox-LDL) may increase plaque instability. Studies have demonstrated that plasma ox-LDL levels and ox-LDL receptor LOX-1 expression are significantly increased in atherosclerotic plaques. This study was designed to observe the role of LOX-1 in modulation of MMPs and the tissue inhibitors of MMPs (TIMPs) and related cellular mechanism(s) in human coronary artery endothelial cells (HCAECs).

Methods and results: HCAECs were cultured and incubated with ox-LDL (10 to 50 mg/ml) for 6 to 24 hours. Ox-LDL increased the expression of mRNA (determined by semi-quantitative RT-PCR) and protein (determined by Western blot) of MMP1 (interstitial collagenase) and MMP3 (stromeolysin 1) in a concentration- and time-dependent fashion. Ox-LDL also moderately increased the expression of TIMP1 and TIMP2 (mRNA and protein). Native-LDL had no effect on the expression of MMPs and TIMP2. These effects of ox-LDL was mediated by its endothelial receptor LOX-1 since pretreatment of HCAECs